International Council for
the Exploration of the Sea

C.M. 1981/L:46, Annex I, II, III.
Biological Oceanography Committee
Ref. Hydrography Committee
Marine Environmental Quality
Committee.

REPORT OF THE WORKING GROUP ON

THE METHODOLOGY OF PRIMARY PRODUCTION,

INCLUDING GUIDELINES FOR THE MEASUREMENT OF PRIMARY PRODUCTION IN THE ICES - AREA

Annex III

REPORT OF THE WORKING GROUP ON THE METHODOLOGY OF PRIMARY PRODUCTION, including guidelines for the measurement of primary production in the ICES - area.

Tvärminne (Finland), 1. - 5. june 1981.

1. The group met at Tvärminne (Finland) on June 1 - 5, 1981. Participants are listed in Annex 1. Dr. Lassig welcomed the delegates on behalf of the Institute of Marine Research (Helsinki) and the Tvärminne Zoological Station of Helsinki University.

   The terms of reference given in the resolution of the Council (C.Res. 198o/2:29) were reviewed to finalize the proposals for measuring primary production with the ^{14}C method began at last years meeting in Texel to discuss alternatives to the ^{14}C method.
   It was noted that this group's activities will be discontinued following the presentation of its report to the Statutory Meeting in 1981.

2. Discussion of the guidelines for the measurement of primary production continued at the point at which they had ceased last year, and began with a reiteration of the groups belief that the establishment of photosynthesis - light relationship (P I curves) is a central issue in productivity assessment. Much attention was therefore given to incubation strategies. It was felt, that the ideal aim might be the description of P I curves for a range of depths in the photic zone, but that since this ideal can rarely be achieved for logistic reasons, the experimenter must choose his incubation strategy in such a way as to either obtain an integrated P I curve for the whole water column or to derive P_{max} or α (gradient of rising limb of P I curve) at each depth. In conjunction with irradiance measurements, the P I curve becomes the tool for the calculation of incorporation rates. In situ, simulated in situ, and artificial light incubation technique were all dwelt upon, as were the techniques available for measuring irradiance. The in situ experiment is regarded as the standard by which other incubation procedures are assessed.

   With regard to technical details, there was general agreement that it is neither desirable nor possible to achieve complete standardization, that different sea areas may require particular modifications of techniques, and that the exact details can be left to the experience and judgement of individual experimenters.

3. The question of data presentation was reviewed; in the context of data banks, it was not clear to participants whether submissions of complete data sets would be acceptable. But it was felt that access through such means to the primary data, as well as to integrated incorporation rates, was desirable.

4. Alternatives to the ^{14}C method were discussed briefly. These are:
   - uptake of eg. ^{13}C, ^{15}N, ^{32}P.
   - utilization of CO_2
   - liberation of O_2
   - track and grain autoradiography
   - DCMU/fluorescence technique.
   - DNA/RNA
   - ETS
   - mitotic figures
   - ATP

   The DCMU/fluorescence method was felt to have some promise since it can be used as a routine survey technique under way, in Norway at least, it has been found to provide estimates comparable to those achieved with ^{14}C. The proposed ATP method, which has not been used, was thought to be worth a trial, though it was pointed out that background levels of ATP might be high.

5. It was noted that ICES has been distributing a description of the liquid scintillation technique applied at the ^{14}C Agency (ref. CM 1980/L:31) following the recommendation made by the WG at its first meeting in 1980 (CM 1980/L:57).

   It was noted that information has been received from Dr. Weichart of the Marine Chemistry Group on the O_2, CO_2 and nutrient changes in water as possible methodological alternatives to the ^{14}C method. This also followed a recommendation made by the WG in 1980 (CM 1980/L:57).

6. It was agreed that the guidelines still needed to be checked on many minor points (see Annex to the recommendation) and tested in the field by means of an intercomparison and intercalibration exercise (cf. terms of reference given in the resolution C.Res. 1978/L:8 establishing the WG). Kristineberg and Aberdeen have been considered as possible places.

ANNEX I : LIST OF PARTICIPANTS AND ADDRESSES.

Mommaerts J.P. (Chairman) :	Unité de Gestion du Modèle Mathématique Mer du Nord et Estuaire de l'Escaut. Ministère de la Santé Publique et de l'Environnement, Manhattan Center, 3 rue des Croisades, 1000 - Bruxelles, Belgium.
Ærtebjerg, G :	The Marine Pollution Laboratory, Kavallergården 6, DK - 2920 Charlottenlund, Denmark.
Berge, G.:	Institute of Marine Research, post box 1870, 5011 Bergen-Nordnes, Norway.
Davies, J.:	Marine Laboratory, P.O. Box 101, Victoria Road, Aberdeen AB9 8DB, United Kingdom.
Gillbricht, M.:	Biologische Anstalt Helgoland, Palmaille 9, D-2000 Hamburg 50, Federal Republic of Germany.
Hansen, Vagn K.:	The Danish Institute for Fishery and Marine Researc Charlottenlund Slot, DK - 2920 Charlottenlund, Denmark.
Lassig, J.:	Institute of Marine Research, P.O. Box 166, SF - 00140, Helsinki 14, Finland.
Lindahl, O.:	Kristinebergs Marinbiologiska Station, 450 34 Fiskebäckskil, Sweden.
Thórdadóttir, T.:	Hafrannsóknastofnunin, Skúlagata 4, Reykjavík, Iceland.
Throndsen, J.:	Institutt for Marinbiologi og Limnologi, Avd. Marin Botanikk, Postbox 1069, Blindern, Oslo 3, Norway.
Wyatt, T.: (Rapporteur)	Fisheries Laboratory, Lowestoft, Suffolk NR33 OHT, United Kingdom.

RECOMMENDATIONS

1. It is recommended that in future primary production measurements made using the ^{14}C method should be based upon the experimental determination of the parameters (productivity index + assimilation number) of the production / light relationship (P/I curve)and that this relationship together with the light attenuation profile, the daily irradiance and some measure of biomass should be used to compute the integrated incorporation over depth and light day periods.

2. It is recommended that this procedure be used for the reevaluation of earlier data.

3. In view of the present problems as to what kind of production is measured by the ^{14}C method, it is recommended that results should be reported simply as ^{14}C incorporation rates, rather than net or gross primary production rates.

4. It is recommended that a number of technical problems in the ^{14}C method (Annex II) be brought to the attention of member countries, and that the individual laboratories be encouraged to investigate these problems, and submit the results to the Biological Oceanography Committee Chairman within a period of 18 months, in order that these results can be utilized during the proposed workshop.

5. It is recommended that, in view of major differences in the strategies employed by individual countries to measure total production, even though using essentially similar techniques, a workshop be arranged for 1 week in 1983, to compare these different measurement strategies. At the same time the workshop would deal with those problems listed in Annex II , which have been investigated, examine the re-evaluations of previous data and revise the ICES guidelines on primary production accordingly.
   It is essential in order to implement this recommendation that a small group be established to plan in detail the 1983 intercomparison workshop.

6. It is recommended that the attention of member countries be drawn to the uncertainties as to what the ^{14}C method measures, to carry out comparisons of the ^{14}C technique with other methods of measuring primary production, and to report the results of these investigations to the Biological Oceanography Committee Chairman.

7. It is recommended that member countries be consulted as to whether they would be willing to submit first level data (ref. to Guidelines) to ICES.

8. In view of the usefulnes of primary production indices (production capacity-, α-, Pmax/chlorophyll a) as tools for environmental quality assessment in the general sense, as well as with respect to man induced effects, the group recommends that the Environmental Quality Committee and the Biological Oceanography Committee jointly reconsider their use in biological monitoring programmes.

ANNEX II : LIST OF PROBLEMS IN THE ^{14}C METHOD.

Problems with the determination of the light penetration profile and the selection of sampling depths.

At the present time a variety of techniques, ranging from the Seechi disc to submersible quantameters, are used to measure the light attenuation profile. There are still questions on how these methods compare. Such effects as changing spectral quality and as varying attenuation coefficient throughout the water column do not facilitate this comparison.

Problems with the incubation.

The effects of bottle size, whether or not to sieve out zooplankton, incubation time, type of light attenuators, light quality, lack of agitation in certain types of incubators, UV radiation - induces errors in certain types of incubation still require attention, although they have been known for quite a time now.

Problems with the diel variation of the parameters of the photosynthesis - light relationship.

This is a problem of fundamental nature if a mechanistic approach using the instantaneous photosynthesis-light relationship is going to replace more traditional approaches involving e.g. full day or half day incubations. The saturated rate of incorporation is known to vary considerably in the course of the day. There is even less information about the possible variations of the slope in the light-proportional range of the photosynthesis-light curve.

Problems at the filtration stage.

Initial respiratory rates that are measured when filtration begins in dim light can be very high. The idea of blocking all metabolic activities with some poison (e.g. lugol) at the end of the incubation (but not in the incubation bottles) has been put forward.

Problems with the preservation of the filters prior liquid scintillation counting

Wetting the filters with HCl (0,1 N) has been proposed as a method preservating as an alternative to deepfreezing and it is then possible to dispense with the HCl fuming step. There is however not enough information about the general validity of this technique.

Problems with preparation of the filters and filtrates for scintillation counting

Preparation for scintillation counting might differ considerably from one author to the other : in some cases filters need to be dissolved in some strong organic solvents before going into the scintillation cocktail whereas in other cases, they will readily dissolve in other kinds of cocktails. Alternatively, filters may be burnt or chemically oxidized and labelled $^{14}CO_2$ collected in some basic solution.

There are questions about the relative amount of aqueous sample that can be accepted by gel-type scintillation liquids. The stability in time of the scintillation efficiency of the gelified sample is also questioned.

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Guidelines for the Measurements of Primary Production in the ICES AREA .

Working Group on the Methodology of Primary Production

Texel (Netherlands), 23 - 25 June 1980
Tvärminne (Finland), 1 - 5 June 1981

Annex III

Guidelines for the Measurement of Primary Production in the ICES AREA.

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I INTRODUCTION

1. These guidelines aim to standardize the methodology of primary production measurements in the ICES area, taking into account as far as possible the diversity of techniques in use at the time of writing. As well as dealing with purely technical aspects, new concepts have been incorporated which modify considerably the current approach to the problem, and which may possibly also alter the daily production figures arrived at. Important considerations stem from the numerous processes assumed to take place in the sea that are relevant to primary production experiments. These are:

   - uptake phenomena:
     - gross photosynthesis
     - dark uptake (anaplerotic reactions, e.g. Wood-Werkman)
   - losses :
     - phytoplanktonic respiration and photorespiration
     - excretion of dissolved organic matter (DOM) by phytoplankton
     - "natural" mortality of phytoplankton
     - grazing mortality of phytoplankton

   Recycling processes, bacterial consumption of produced DOM and bacterial dark uptake are also important processes.

Respiration and internal recycling processes are problems which perhaps deserve more attention than hitherto. Indeed the problem of net versus gross production estimates is central to an operational definition of productivity (10). This is indicated by the frequent observation that differences between community respiration and autotrophic production lead to unbalanced carbon budgets (7). There is evidence that on some occasions autrotrophic production is too low to balance the heterotrophic processes measured, as well as exchanges at the boundaries. Whether it is the gross primary production figures that are underestimated, the respiration figures that are overestimated, or other organic carbon pathways that have been overlooked is still a problem. Some authors (6) are accumulation evidence that phytoplankton itself could be responsible for a much larger part of the total respiration than previously assumed. However, this kind of conclusion strongly depends on the methods used. In this context, incubation times could play a determining role. Indeed, the pattern of ^{14}C flow through the various compartments (algal, bacterial, etc.) of the system will vary with time. Region, time of the year and community composition can play important roles as well. At the present time, the respiration problem appears far from being settled. This problem deserves more attention in the future, since accurate esti-

mates of the respiration rate of phytoplankton as well as of heterotrophic organisms are necessary for a correct understanding of ecosystem functioning, and in the interpretation of primary production measurements.

2. It was therefore recognised by the Working Group that, in view of the present state of uncertainty concerning the definition of measured rates, results should be presented in a more objective way, and should provide ^{14}C incorporation rates rather than gross or net production rates.

3. In view of the advantages to be gained from insights into the phenomena involved in photosynthesis, particularly as reflected by the behavior of such biologically significant parameters as the rate of change in photosynthetic rates at increasing but low light intensities, and maximum photosynthetic rates, it was agreed that knowledge of the photosynthesis-light relationship (the P I curve) is required for the compilation of integrated incorporation rates over depth and light day periods.

4. A first section of these guidelines is devoted to concepts, incubation strategies and calculations related to the P I curve.
The second section is devoted to more technical aspects. With respect to these the group decided that it was not possible to effect a complete standardization of methods, since each area might require a modification of these, and resources differ from one laboratory to another; the exact details can be left to the intelligence and experience of the operator. Various points which it is thought require judgement are pointed out in this 2^{d} section (chapt. III), and references given.

II APPROACHES BASED ON THE PHOTOSYNTHESIS-LIGHT RELATIONSHIP.

1. Concepts and strategies for sampling and incubation.

Sampling and incubation strategies will tend to comprise an optimal scheme taking into account conflicting objectives i.e. monitoring needs (often implying coverage of very large areas within a short time) and precision needs (implying a more careful description of the production profile whenever local particularities justify it).

Thus there are basically two types of experiments to consider:

1) that which allows a complete characterization of the photosynthesis-light relationship (the P I curve) for each of the relevant layers at a given station (one or two usually).
   The P I curve is meant to be used as a calculating tool for extrapolation to whole day (i.e. light-day) production. Moreover, characteristic parameters such as initial slope (rate per unit light at low light intensity) and saturated rate are valuable physiological and environmental indexes when normalized to chlorophyll.
   Several incubation techniques can be used to derive a P I curve: in situ incubations and simulated in situ either under natural daylight or artificial light. The simulated in situ techniques ought to be calibrated against in situ incubations.

2) that which allows interpolation between stations where type 1 experiment is carried out. Adopting this approach, it is assumed that the P I curve normalized to phytoplankton standing stock, will not change significantly for a given area and a given time during the survey cruise.
   Phytoplankton standing stock, or a related parameter, can then be measured at the recommended depths (see § 6 in chapter III) instead of undertaking a full primary production exercise.
   The parameter measured could be chlorophyll a or, alternatively, one of the two characteristic parameters of the non-normalized P I curve (either the rate of change of incorporation per unit light or the saturated rate). In the latter case, samples are incubated at a single light intensity using the light incubator.

Rem: there are definite advantages in using the parameters of the P I curve since the sensitivity is high and the values measured have also an environmental meaning, especially when expressed per unit chlorophyll a. There is however considerable riscs in using

these parameters in a survey since diel variation - especially that of the saturated rate - is an established fact (4,8). This ought to be taken into account in the calculations of integral production (e.g. (4,9)).

In all cases, the incubation time should be the same for in situ, deck and artificial lightincubation. It should be between 2 and 4 hours.

2. Calculation of light-day carbon incorporation (particulate + dissolved) at a given station, using the P I curve. (see also example given at the end of this chapter).

2.1 The production (mg C $m^{-3}h^{-1}$) versus light curves from that station or from the nearest reference station in the area are normalized to the chosen index of standing stock (chlorophyll, saturation incorporation rate or rate change per unit light) and plotted.

2.2 The light field - depth versus time - for the whole day is calculated using the light penetration profile and the day course of 100% irradiance, and tabulated. Similarly, the standing stock field - depth versus time - is tabulated. Although the latter might reduce to a single profile in the simplest cases, provision should be made here for a possible diel variation of the parameter chosen to serve as an index of standing stock.

2.3 The normalized production - light curves are used to convert all the light values of the depth-time field into incorporation values normalized to standing stock. Finally these values are transformed into true production values (mg C m^{-3} h^{-1}) by multiplying them by the standing stock values from the corresponding depths and times.

2.4 The calculation of daily incorporation rate (mg C $m^{-2}day^{-1}$) can be done in several ways once the table of time versus depth production values has been established. It is largely a matter of interpolation and integration techniques, both more or less empirical.

2.5 a) These operations - especially (3) and (4) - are lengthy. They are best managed with a small computer (e.g. the desk top type).

b) The experimental production-light profile can be simulated by a variety of mathematical functions. Hence, the computing work is greatly facilitated. The most appealing formulae are those that are precisely parametrized by the maximal incorporation rate and the

change of rate per unit light at low light intensity (9,11).

3. Results to be reported.

3.1 Primary production, chlorophyll and light data need to be reported. There are basically three levels of primary production data to be considered:

1) First-level data refer to light bottle, dark bottle and zero-time incorporation rates, calculated as in § 12 of Chapter III, for particulate and dissolved production from the different incubations. They all ought to be reported separately, together with the corresponding light intensities.

2) Second-level data refer to more synthetic information as stems from the P I curve. The figures which should be reported are:
   - the saturated rate of incorporation (p_{max}) from the best fitted curve (mg C $m^{-3}h^{-1}$)
   - the corresponding light intensity I_{max}
   - the rate (α) of change of the incorporation per unit light in the proportional range, from the best fitted curve (mg C $m^{-3}h^{-1}I^{-1}$)
   - the rate of apparent loss (r) measured at light intensity zero (extrapolation of the tangent to the y-axis)(mg C $m^{-3}h^{-1}$)

Rem: from this stage on, the rates refer to total incorporation(particulate + dissolved) and are corrected for dark uptake.

3) Third-level data refer to incorporation integrated over the light-day and the water column (mg C $m^{-2}day^{-1}$).

3.2 Chlorophyll profiles, light penetration profiles and day courses of 100% irradiance need to be reported.

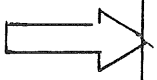
4 Detailed example of calculation (based on fictive results from an in situ incubation around 1o a.m.)

4.1 production profile

depth (m)	light bottle (mg C $m^{-3}h^{-1}$)	dark bottle (mg C $m^{-3}h^{-1}$)	difference (mg C $m^{-3}h^{-1}$)
o	12.06	-	11.20
0.65	15.68	-	14.82
1.50	18.62	-	17.76
3.00	15.94	0.86	15.08
5.00	7.16	-	6.30
10.00	1.52	-	0.66

4.2 chlorophyll a profile and interpolation.

depth (m)	chlorohyll a measured (mg m^{-3})
0	4
0.65	3.9
1,50	3.7
3.00	2.9
5.00	1.5
10.00	1.1



depth (m)	chlorophyll interpolated (mg m^{-3})
0	4
1	3.85
2	3.45
3	2.90
4	2.15
5	1.50
6	1.30
7	1.20
8	1.15
9	1.1
10	1.1

4.3 light penetration profile : calculated from an attenuation coefficient $\eta = 0.45\ m^{-1}$

depth (m)	light intensity (%) at selected sampling depth
0	100
0.65	75
1.50	50
3.00	25
5.00	10
10.00	1

depth (m)	light intensity (%) in the 10 m profile
0	100
1	63
2	40
3	25
4	16
5	10
6	6
7	4
8	3
9	2
10	1

4.3 actual light intensity during the incubation (based on an average value of surface irradiance $I_o = 6$)

depth (m)	light intensity (arbitrary units)
0	6
0.65	4.5
1.50	3
3.00	1.5
5.00	0.6
10.00	0.06

4.4 Surface light evolution during the day : read from e.g. continuous recording

hour	light intensity (= irradiance at 100% = I_o) (arbitrary units)
6	0
7	1.7
8	1.4
9	3.5
10	6.0
11	7.3
12	3.6
13	4.0
14	4.0
15	2.0
16	2.5
17	1.0
18	0

depth (m)	hour												
	6	7	8	9	10	11	12	13	14	15	16	17	18
0	0	1.70	1.40	3.50	6.00	7.30	3.60	4.00	4.00	2.00	2.50	1.00	0
1	0	1.07	0.88	2.21	3.79	4.61	2.27	2.53	2.53	1.26	1.58	0.63	0
2	0	0.68	0.56	1.39	2.39	2.91	1.43	1.59	1.59	0.80	1.00	0.40	0
3	0	0.43	0.35	0.88	1.51	1.84	0.91	1.01	1.01	0.50	0.63	0.25	0
4	0	0.27	0.22	0.56	0.95	1.16	0.57	0.64	0.64	0.32	0.40	0.16	0
5	0	0.17	0.14	0.35	0.60	0.73	0.36	0.40	0.40	0.20	0.25	0.10	0
6	0	0.11	0.09	0.22	0.38	0.46	0.23	0.25	0.25	0.13	0.16	0.06	0
7	0	0.07	0.06	0.14	0.24	0.29	0.14	0.16	0.16	0.08	0.10	0.04	0
8	0	0.04	0.04	0.09	0.15	0.18	0.09	0.10	0.10	0.05	0.06	0.03	0
9	0	0.03	0.02	0.06	0.10	0.12	0.06	0.06	0.06	0.03	0.04	0.02	0
10	0	0.02	0.o1	0.04	0.06	0.07	0.04	0.04	0.04	0.02	0.03	0.01	0

4.5 Table of depth versus time light field, computed from the daily course of of I_o and the formular

$$I_d = I_o \, e^{-\eta d} \quad \text{(with } \eta = 0.46\text{)}$$

or combining the daily course of I_o with the actual light penetration (%) profile if the Lambert law is not obeyed.

4.6 Normalization of the production profile to chlorophyll a.

Light (I)	Production	Chlorophyll a	$\frac{\text{Production}}{\text{chlorophyll a}} = k$
6	11.20	4	2.8
4.5	14.82	3.9	3.8
3	17.76	3.7	4.8
1.5	15.08	2.9	5.2
0.6	6.30	1.5	4.2
0.06	0.66	1.1	0.6

Plotting k in function of I generates the P I curve (fig.) which will serve as an extrapolating tool for the calculation of daily integrated production.

Therefore, values of k are read on the curve for each value of I_d, hence creating the table of the next §.

Rem: As this is particularly tedious, a mathematical function adjusted to the experimental P I curve will help to compute k's instead of reading them.

Such function utilizes the parameters k_{max} and α measured on the experimental P I curve (see fig.) The fitted mathematical function is also shown in this figure. A perfect agreement between both experimental and mathematical curves is seldom achieved.

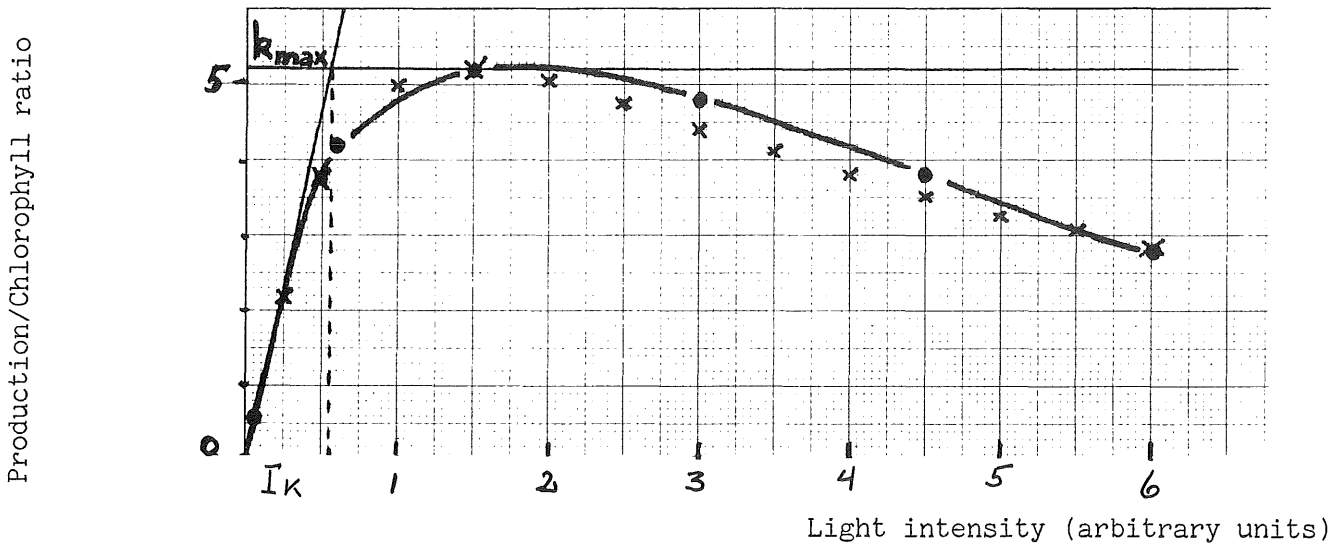


Fig. Production/Chlorophyll versus I plot.

Characteristic parameters : $k_{max} = 5.20$

$I_k = 0.55$

$\alpha = k_{max}/I_k = 9.45$

• —— • experimental

× × × model fitting (additional parameters : b = 1.22, γ = o.22 and n = 1 (9))

depth \ hour	6	7	8	9	10	11	12	13	14	15	16	17	18
0	0	5.15	5.20	4.09	2.86	2.44	4.03	3.79	3.79	5.03	4.74	5.01	0
1	0	5.07	4.85	4.92	3.92	3.46	4.88	4.72	4.72	5.18	5.18	4.25	0
2	0	4.41	4.00	5.20	4.81	4.47	5.20	5.18	5.18	4.70	5.01	3.22	0
3	0	3.39	2.91	4.85	5.20	5.10	4.89	5.02	5.02	3.74	4.25	2.21	0
4	0	2.36	1.97	4.00	4.95	5.13	4.04	4.29	4.29	2.72	3.22	1.47	0
5	0	1.56	1.29	2.91	4.15	4.54	2.98	3.22	3.22	1.81	2.21	0.93	0
6	0	1.03	0.84	1.97	3.10	3.55	2.05	2.21	2.21	1.21	1.47	0.56	0
7	0	0.66	0.56	1.29	2.13	2.51	1.29	1.47	1.47	0.75	0.93	0.38	0
8	0	0.38	0.38	0.84	1.38	1.64	0.84	0.93	0.93	0.47	0.56	0.28	0
9	0	0.28	0.19	0.56	0.93	1.12	0.56	0.56	0.56	0.28	0.38	0.19	0
10	0	0.19	0.09	0.38	0.56	0.66	0.38	0.38	0.38	0.19	0.28	0.09	0

4.7 Table of k's in the time-depth field, calculated from the P I curve and the time-depth light field (table from § 4.5)

depth \ hour	6	7	8	9	10	11	12	13	14	15	16	17	18	Σ
0	0	20.60	20.80	16.36	11.44	9.76	16.12	15.16	15.16	20.12	18.96	20.04	0	184.52
1	0	19.52	18.67	18.94	15.09	13.32	18.79	18.17	18.17	19.94	19.94	16.36	0	196.91
2	0	15.21	13.80	17.94	16.59	15.42	17.94	17.87	17.87	16.22	17.28	11.11	0	177.25
3	0	9.83	8.44	14.07	15.08	14.79	14.18	14.56	14.56	10.85	12.33	6.41	0	135.10
4	0	5.07	4.24	8.60	10.64	11.03	8.69	9.22	9.22	5.85	6.92	3.16	0	82.64
5	0	2.34	1.94	4.37	6.23	6.81	4.47	4.83	4.83	2.72	3.32	1.40	0	43.26
6	0	1.34	1.09	2.56	4.03	4.62	2.67	2.87	2.87	1.57	1.91	0.73	0	26.26
7	0	0.79	0.67	1.55	2.56	3.01	1.55	1.76	1.76	0.90	1.12	0.46	0	16.15
8	0	0.44	0.44	0.97	1.59	1.89	0.97	1.07	1.07	0.54	0.64	0.32	0	9.94
9	0	0.31	0.21	0.62	1.02	1.23	0.62	0.62	0.62	0.31	0.42	0.21	0	6.19
10	0	0.21	0.10	0.42	0.62	0.73	0.42	0.42	0.42	0.21	0.31	0.10	0	3.96
Σ	0	75.66	70.40	86.40	84.89	82.61	86.42	86.55	86.55	79.23	83.15	60.30	0	882.16

8 Table of primary production values in the time-depth field, calculated from the values of k (table of § 4.7) and the chlorophyll or profile (§ 4.2). Values in the core of the table are in mg C $m^{-3}h^{-1}$.
The sums of the rows are mg C $m^{-3}day^{-1}$ and the sums of the columns are mg C $m^{-2}h^{-1}$. The total sum is of course the daily integrated production value in mg C $m^{-2}day^{-1}$.

Rem: having used from the beginning (4.5) a matrix with a time step = 1 hour and a depth step = 1 meter provides a fairly good approximation of the true integral production. However, other techniques are equally valid and perhaps less time consuming, especially if there is no computer available. Other detailed examples can be found in e.g. G.Ærtebjerg's report on the Danish standard (1980) or in Mommaerts (1981) (9,22).

III TECHNICAL ASPECTS OF THE ^{14}C METHOD.

1 Preparation of ^{14}C solution.

1.1 Ampoules can be purchaed from the International Agency for ^{14}C Determination in Denmark or obtained from other sources. Alternatively, the solution used in the production studies should be prepared from $Ba^{14}CO_3$ transferred to $Na^1H^{14}CO_3$ in a closed evacuated system by acidification of the $Ba^{14}CO_3$ and absorption of the evolved $^{14}CO_2$ in a NaOH solution (13).

1.2 The active solution should be diluted with freshly prepared double glass destilled water.

1.3 The pH of the solution should be adjusted to 9.5 - 10.0. The pH range is chosen to minimize loss of ^{14}C during storage and handling of the solution and should not effect either the partial pressure of CO_2 or the photosynthesis of algae in sea water.

1.4 Only high grade (p.a.) chemicals should be used for preparation of the ^{14}C solution.

2 Standardization of ^{14}C solution in the ampoules.

Liquid scintillation counting can conveniently be used as a basis for computation of the absolute radioactivity.
However, for the purpose of intercalibration, ampoules (preferably 10 chosen randomly from the batch) should be sent to the International Agency for ^{14}C Determination. Experience has shown that this procedure should be repeated each time a new source of $Ba^{14}CO_3$ is used, and each time the preparatory technique is changed.

3 Samplers and bottles.

3.1 Non-transparent, non-toxic sampling devices must be used. Experimental bottles should be thoroughly cleaned regularly, to meet similar standards to those required for culture flasks. They can however, if necessary, be re-used a few times, provided they are given the usual HCl treatment and the normal rinsing steps.

3.2 For practical purposes, bottle size can range between 25 to 100 cm^3 whenever simulated in situ or artificial light incubation is considered.

Larger vessels may be required for in situ incubations in specific

situations (5). The bottles should be made of ligh-quality hard glass (e.g. Jena).

3.3 Before the start of the experiment the bottles should be rinsed with water from the appropriate sample. The bottles should be filled up to the neck, leaving an air bubble. The stoppers of the bottle should always be tightly closed in order to avoid less of ^{14}C during the experiment.

4 Concentration of ^{14}C solution.

The ^{14}C solution should be added to the experimental bottles in such concentration that statistically sufficies estimations of the radioactivity fixed by photosynthesis in the different fractions of the sample (dissolved and particulate) can be obtained. However, it is also important not to disturb the CO_2 equilibrium in the water sample by adding too much $NaH^{14}CO_3$ solution.

5 Dark fixation and non-biological fixation of carbon.

Fixation measured in the dark is due to biological and non-biological mechanisms. The biological mechanisms are associated to the tricarboxylic cycle (e.g. the Wood-Werkman reaction), and hence also occur in bacteria and zooplankton. Non-biological phenomena are related to adsorption, contamination and to back ground sensu stricto.

Dark fixation of carbon should be reported separately from the light bottle fixation.
Zero-time incorporation is thought to measure the non-biological processes. This should be determined whenever possible.
At each station at least, one dark bottle should be used. If vertical inhomogeneities of dark fixation are suspected (for example if the chlorophyll is inhomogeneously distributed) more dark incubations should be performed at the relevant depths.

6 Sampling depths.

Sampling depths should be selected so as to give adequate coverage of the photosynthesis-depths profile. It is therefore recommended that sampling depths be selected from those standard oceanographic depths which are as close as possible to depths at which e.g. 100%, 75%, 50%, 30%, 10%, 3% and 1% of the subsurface levels are found.

7 Irradiance and light penetration profile.

7.1 Irradiance should be expressed in 10^{18} quanta $m^{-2}s^{-1}$ (or 10^{5} quanta $cm^{-2}s^{-1}$) or Joules $m^{-2}h^{-1}$. Whenever possible, measurements should be performed with a quantameter operating in the range 350/400 - 700 nm. Other types of instruments (e.g. pyranometers) might be used provided an appropriate calibration with the quantameter has taken place. The time course of irradiance during the whole light-day should be recorded on each occasion.

7.2 Light penetration profiles ranging from subsurface irradiance (taken as 100%) to 1% irradiance should be established with a quantameter operating in the range 350/400 - 700 nm whenever possible. Other devices might be used (e.g. submarine photometer equiped with a green filter and a diffusing filter; Secchi disc), provided an appropriate calibration with a quantameter has been carried out. It must be noted that the relation of log (I%) to depth is not always linear, especially in the 60% - 100% irradiance range.

8 Incubation techniques.

8.1 In situ

In this technique, samples are resuspended at standard depths (see § 6) after ^{14}C inoculation. In doing this, special care must be taken to avoid excess light. This method has been described in many papers (for a review see (15,16,17)).

8.2 Simulated in situ (deck incubator)

Samples are placed in an incubator cooled by surface seawater and exposed to daylight. Light levels in the incubator are controlled by means of neutral filters (e.g. black gauze). Some precautions must be taken in order to eliminate UV radiation (either using special glass plates or managing a sufficiently thick water layer above the incubation bottles) and avoid shadows (either from the incubator walls or ship superstructures). The attenuation coefficients of the neutral filters should be determined with a quantameter in the operating incubator (i.e. with the cooling water).

8.3 Artificial light incubators.

Such incubators have been described by several authors (e.g.(14)). Philips TLD 33 fluorescent tubes are usually used and the maximal irradiance normally allows saturation rates to be measured. Lower light

levels are managed with the aid of neutral filters. The bottles are usually fixed on a rotating wheel that provides adequate agitation. Cooling is provided either by circulating surface seawater or with a refrigeration system.

9 Filter and filtrate procedure.

9.1 Filter and filtrate collection

9.1.1 Water samples should be filtered immediately after the production experiment has been stopped, in order to avoid less of ^{14}C due to respiration. Filters with even distribution of pore size, and good solubility with respect to scintillation liquids are prefered. Pore size should not exceed 0.5 µm (19). Filters should be wetted before filtration starts. The suction pressure should not exceed 0.3 kp cm^{-2}. The whole filtration procedure should not exceed 0.5 hour for the entire series of bottles. If this is unavoidable, subsampling is recommended since this will also prevent self-absorption or quenching at the counting stage. The filters need normally not to be washed but, whenever bottles and filtration funnels need to be rinsed, this should occur at the end of the filtration procedure but before the last cm^3 has passed through the filter.

9.1.2 For practical reasons, filtrates will often be subsampled. Therefore, the ratio of the subsample volume to initial volume must be known.

9.2 Preservation of filters and filtrate samples.

9.2.1 When scintillation counting is used, the filters can be introduced to the empty scintillation vials and then deepfrozen.
When Geiger counting is used, filters are dried in the presence of freshly dried silicagel in order to ensure rapid dessication.

If these procedures are not possible, the filters should be exposed to formalin vapors to stop all biodegradation mechanisms.

9.2.2 Filtrate subsamples should either be deep-frozen or acidified (pH = 2). The scintillation vials are adequate containers.

9.3 Preparation of filters and filtrate samples.

9.3.1 The filters should be exposed to HCl fumes for 5 to 10 minutes prior Geiger counting or further preparation for scintillation counting.

9.3.2 The filtrate should be acidified to pH = 2 and bubbled within the

scintillation vial until all inorganic labelled ^{14}C has been eliminated (no more variation in the radioactivity level) (12,18). The bubbling time will depend on the experimental set-up and should therefore be determined on a test sample.

9.4 Total sample method.

It is possible to acidify and bubble in total incubated water samples in the same way filtrates are treated, hence eliminating the filtration stage (12).
Results are expressed as total ^{14}C incorporation. This simplified method might prove useful when speed is a more important factor in the sampling strategy than the completeness of information. It is useful to intercalibrate this technique with the [filter + filtrate] technique.

10 Counting.

Various counting techniques are nowadays available, ranging from Geiger counting and proportional counting to liquid scintillation counting.
The efficiency of the counting, i.e. the ratio of counts per minute (cpm) to disintegrations per minute (dpm) varies considerably according to the techniques. Liquid scintillation is the most efficient technique and has the highest versatility. In all cases, the efficiency of the counting technique should be known, including the relation of efficiency to the amount and nature of filtered material (self- absorption or quenching problems).
There are numerous and specific problems with all methods, such as
: - with Geiger counting : window thickness, self-absorption, geometry, air pressure, humidity, etc.
: - with scintillation counting : solubility of samples in cocktail, quenching, initial chemoluminescence implying some delay before counting, nature of vial conditionning storage time, etc.
Therefore, it is strongly recommended to consult specialized publications for further details (2o,21).

11 Total CO_2 concentration.

11.1 Carbon dioxide concentration can be calculated from carbonate alkalinity, temperature, pH and chlorinity. The relation between these variables is well established for the Baltic and the North

Sea (1,2,3).

The carbonate alkalinity - total CO_2 conversion factor can be read either from the original graphs of Buch or from tables published in standard books (e.g.(17)).

11.2 Carbonate alkalinity is calculated from total alkalinity. This concerns a correction for the presence of boric acid, usually assuming that the free boric acid is always present in seawater at a constant ratio to chlorinity. In the Baltic area, the concentration of H_2BO_3 is about 1% of total alkalinity.

11.3 The ratio of total alkalinity to chlorinity is well known for the Baltic area (2,4). On the other hand, it is known to be fairly constant in most sea areas (= o.123). Practically, alkalinity need to be experimentally determined only in coastal areas with marked land drainage, and at great depths.

12 Calculation of carbon uptake.

Carbon incorporation is calculated separately for each fraction using the following equation:

$$\frac{\text{incorporated radioactivity (dpm)}}{\text{added radioactivity (dpm)}} \times 1.05 = \frac{\text{incorporated carbon}}{\text{available carbon}}$$

- "incorporated radioactivity" refers to disintegrations per minute. Therefore, counts per minute (cpm) must be converted into dpm, using the efficiency of the counting technique as the conversion factor. If a subsample is counted, this radioactivity is of course multiplied by the ratio of the total incubated volume to the subsampled volume.
- "added radioactivity" also refers to dpm. The absolute radioactivity of an ampoule can be determined by liquid scintillation counting or be standardized at the International Agency for ^{14}C Determination.
- "available carbon" refers to total CO_2 concentration in the experimental water, in the same units as incorporated carbon (mg C m^{-3}).
- The factor 1.05 corrects a 5% difference between ^{14}C and ^{12}C uptake rates.

It is recommended that further correction factors (e.g.respiration) are not introduced at the present stage.

IV

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